Experimental studies revealed that the N-terminal (1-18) fragment is responsible for the activity of the whole peptide, with a negligible toxicity towards eukaryotic cells, thus representing an excellent canditate for future applications. It is expected, like most of the known AMPs, to target the bacterial plasma-membrane but its 3D-structure and detailed mode of action are still unknown. Before an in-depth investigation on peptide/membranes interactions could be undertaken, it is necessary to characterize its folding propensity in solution, in order to understand what is intrinsically due to the peptide sequence and what is actually driven by the membrane interaction. In this scenario, the present study represents the first structural investigation on Esculentin-1b(1-18). Liquid state NMR was employed to determine the peptide structure, moving from water to increasing amounts of trifluorethanol. NMR parameters have been used as restraints during structure determination through a simulated annealing procedure. The results showed that Esculentin-1b(1-18) has a clear tendency to fold in a helical conformation with increasing the environment hydrophobicity, confirming circular dichroism data. Interestingly, the helix is formed only for residues ranging from 3 to 11, while it appears to be unstructured in the rest of the peptide. Nevertheless, the whole conformation was found to be amphipathic with a 3-mer hydrophobic cluster right in the middle of the unstructured segment, which might act as an anchoring tail upon membrane binding.

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Membrane Disruption by Antimicrobial Peptide Protegrin-1 is Tuned by Incorporation of Cholesterol and Phosphoethanolamine Lipids

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Antimicrobial peptides (AMPs) are a class of small (less than 100 amino acid residues) host defense peptides that induce selective membrane lytic activity against microbial pathogens. To understand the mechanism of membrane disruption by AMPs, we investigated, via atomic force microscopy, topological changes induced by protegrin-1 (PG-1), an 18-residue, cationic, β-sheet AMP isolated from pig leukocytes, in supported phospholipid bilayers (SPBs). Lipid mixtures of dioleoylphosphatidylserine (DOPS), dioleoylphosphatidylcholine (DOPC), and cholesterol were used to mimic eukaryotic cell membranes while bacterial cell membranes were emulated by substitution of cholesterol for dioleoylphosphatidylethanolamine (DOPE). We have previously shown that AMP disruption of zwitterionic dimyristoylphosphatidylcholine (DMPC) SPBs induce concentration dependent structural transformations that progress from fingerlike instabilities at bilayer edges, to the formation of sievelike nanoporous structures, and finally to a network of wormlike micellar structures. The observed transformations suggest that the peptides act to lower the interfacial energy of the bilayer in a manner similar to detergents. Detergent solubilization of membranes encompass processes such as pore formation, blebbing, budding, and vesiculation that share common saddle-splay (''negative Gaussian'') curved topologies. We pose that membranes rich in negative curvature lipids such as those with phosphoethanolamine (PE) headgroups enhance the efficacy of AMP disruption while those membranes containing cholesterol retard disruption. Results have shown that cholesterol incorporation shifts the disruption susceptibility of a bilayer to a higher peptide dosage regime while an opposite effect is observed in the presence of PE. The observed trend sheds light on a lingering debate as to how nature has evolved AMPs to discriminate between host and pathogen.

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Effect of Head Group and Curvature on Binding of the Antimicrobial Peptide Tritrpticin to Lipid Membranes

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In this work we examine the interaction between the 13-residue cationic antimicrobial peptide (AMP) tritrpticin (VRRFPWWWPFLRR, TRP3) and model membranes of variable lipid composition. The effect on peptide conformational properties was investigated by means of CD (circular dichroism) and fluorescence spectroscopies. Based on the hypothesis that the antibiotic acts through a mechanism involving toroidal pore formation, and taking into account that models of toroidal pores imply the formation of positive curvature, we used large unilamellar vesicles (LUV) to mimic the initial step of peptide-lipid interaction, when the peptide binds to the bilayer membrane, and micelles to mimic the topology of the pore itself, since these aggregates display positive curvature. In order to more faithfully assess the role of curvature, micelles were prepared with lysophospholipids containing (qualitatively and quantitatively) head groups identical to those of bilayer phospholipids. CD and fluorescence spectra showed that, while TRP3 binds to bilayers only when they carry negatively charged phospholipids, binding to micelles occurs irrespective of surface charge, indicating that electrostatic interactions play a less predominant role in the latter case. Moreover, the conformations acquired by the peptide were independent of lipid composition in both bilayers and micelles. However, the conformations were different in bilayers and in micelles, suggesting that curvature has an influence on the secondary structure acquired by the peptide. Fluorescence data pointed to an interfacial location of TRP3 in both types of aggregates. Nevertheless, experiments with a water soluble fluorescence quencher suggested that the tryptophan residues are more accessible to the quencher in micelles than in bilayers. Thus, we propose that bilayers and micelles can be used as models for the two steps of toroidal pore formation.

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Impact of Aminoacylated Phospholipids on the Interactions of Antimicrobial Peptides with Phospholipid Vesicles

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Aminoacylated phosphatidylglycerols (PG) are common lipids in the cytoplasmic membranes of Gram-positive bacteria. Their presence in staphylococcal membranes has been linked to increased resistance to a number of antibacterial agents, including antimicrobial peptides. Most commonly, the PG headgroup is esterified to lysine, which converts anionic PG into a cationic lipid with a considerably increased headgroup size. In the present work, we investigated the interactions of two well-studied antimicrobial peptides, cecropin A and mastoparan X, with lipid vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG), containing varying fractions of an aminoacylated phosphatidylethanolamine (PE), a stable analog of the corresponding PG-derivative. To differentiate between the effects of headgroup charge and size on peptide-lipid interactions, we synthesized two different derivatives. In one, the headgroup was modified by the addition of lysine, and in the other, by glutamine. The modification by glutamine results in a phospholipid with a headgroup size comparable to that of lysylated version. However, whereas lysyl-PE is cationic, glutamyl-PE is zwitterionic. We found that as long as the concentration of aminoacylated PEs did not exceed ~30 mol%, binding of either peptide was not significantly altered. These observations are understood through the interplay between lipid charge and headgroup size and their effect on membrane binding and peptide-induced release of content from lipid vesicles.

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Effect of Electrostatic Interactions on the Membrane Interactions of Amphiphilic Peptides with Antimicrobial Potential

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A wide variety of organisms produce antimicrobial peptides as part of their first line of defense. These short cationic peptides are being considered as a new generation of antibiotics and represent great hopes against multiresistant bacteria which are an important clinical problem. Despite their diversity, antimicrobial peptides generally share common characteristics such as a short length of amino acids, a positive charge and an amphiphilic character. Also, it is important to note that the main target of antimicrobial peptides is the membrane(s) of pathogens. We have previously shown that a non-natural peptide composed of 14 residues (10 leucines and 4 phenylalanines modified with a crown ether) has a helical secondary structure, and is able to disrupt lipid bilayers but is not selective towards bacterial membranes. To gain specificity against negatively charged membranes, several leucines of this 14-mer have been substituted by positively charged residues (lysine, arginine, histidine). In addition, we have compared the results with those obtained with peptides substituted with negatively charged residues. Solid-state NMR experiments performed in model membranes and lipids oriented between glass plates were used to better characterize the mode of action of the charged peptides. In addition, It has been possible to determine the orientation of the charged peptides relative to the bilayer normal by using attenuated total reflection spectroscopy. Complementary results have also been obtained by infrared and fluorescence spectroscopy.